

Appendix A
Overview of Changes to the Copper Protocol

1. **Test Microbes and Culture Preparation:** *Enterobacter aerogenes* as a Gram negative bacterium was not deemed essential to support the sanitizer claim and was removed from the protocol. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were retained. The preparation of test cultures was revised and is now consistent with the OECD Quantitative Method for Bacteria; Tryptic Soy Broth is used as the growth medium. (Refer to EPA Standard Operating Procedure MB-25: OECD Quantitative Method for Evaluating Bactericidal Activity of Microbicides Used on Hard, Non-Porous Surfaces)
2. **Quality Control:** A statement indicating that all aspects of testing must be conducted using Good laboratory Practice Standards was added to the protocol.
3. **Copper Product Attributes:** Observations of surface characteristics may be indicated as qualitative and/or quantitative assessments.
4. **Abrasion/Exposure Cycles:** The abrasion/exposure cycles were revised to include an 8 week period rather than a 12 week period. In addition, the initiation of the efficacy evaluation was revised to state with three days of the last abrasion/exposure cycle.
5. **Details for Supplies and Materials:** Sources have been provided for several reagents including the sodium hypochlorite, abrasion boat, spray bottle and the lint free cloth. The protocol has been revised to replace the abrasion pad on a daily basis, and 95-98% ethanol has been specified for use in the preparation of test carriers.
6. **Soil Load:** A three-part soil load has been specified as an addition to the test inoculum prior to carrier inoculation. The three-part soil load is consistent with the soil load requirement in the OECD Quantitative Method for Bacteria and serves to represent the soil burden found on environmental surfaces.
7. **Continuous Claim:** The protocol as written can be used to support a “continuous reduction” claim.
8. **Contact Time:** The revised protocol provides for a contact time of 1 hour, and for less than one hour upon consultation with EPA.
9. **Chelating Agent Activity:** The potential effects of chelating materials on copper/copper-alloy surfaces is under consideration.